NEURAL REGENERATION RESEARCH
January 2016, Volume 11, Issue 1
www.nrronline.org

RESEARCH ARTICLE

Specific effects of c-Jun NH2-terminal kinase-interacting protein 1 in neuronal axons

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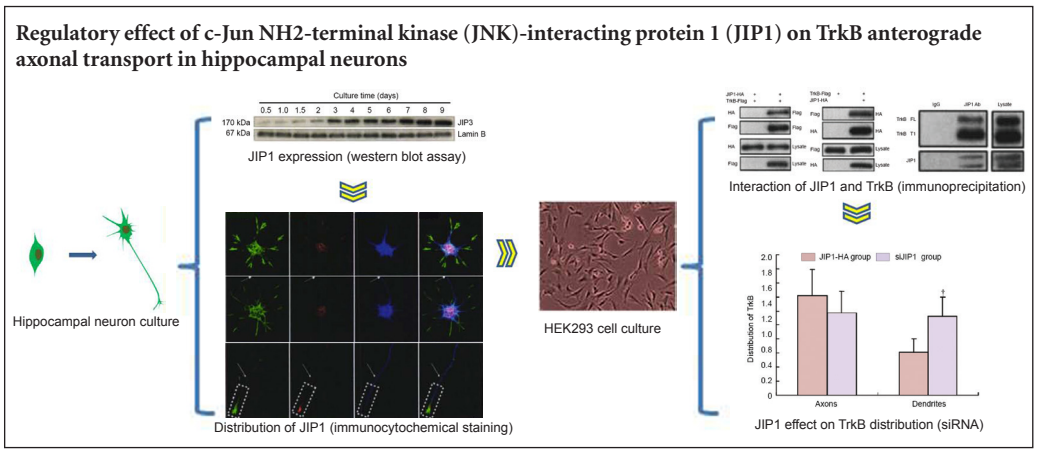
How to cite this article: *Tang S, Wen Q, Zhang XJ, Kan QC (2016) Specific effects of c-Jun NH2-terminal kinase-interacting protein 1 in neuronal axons. Neural Regen Res 11(1):114-118.*

Funding: *This study was supported by the Henan Province Education Department Key Project of Science and Technology Research in China, No. 12A350006.*

Graphical Abstract



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doi: *10.4103/1673-5374.175055*
http:*//www.nrronline.org/*

Accepted: *2015-10-26*

Abstract

c-Jun NH2-terminal kinase (JNK)-interacting protein 3 plays an important role in brain-derived neurotrophic factor/tropomyosin-related kinase B (TrkB) anterograde axonal transport. It remains unclear whether JNK-interacting protein 1 mediates similar effects, or whether JNK-interacting protein 1 affects the regulation of TrkB anterograde axonal transport. In this study, we isolated rat embryonic hippocampus and cultured hippocampal neurons *in vitro*. Coimmunoprecipitation results demonstrated that JNK-interacting protein 1 formed TrkB complexes *in vitro* and *in vivo*. Immunocytochemistry results showed that when JNK-interacting protein 1 was highly expressed, the distribution of TrkB gradually increased in axon terminals. However, the distribution of TrkB reduced in axon terminals after knocking out JNK-interacting protein 1. In addition, there were differences in distribution of TrkB after JNK-interacting protein 1 was knocked out compared with not. However, knockout of JNK-interacting protein 1 did not affect the distribution of TrkB in dendrites. These findings confirm that JNK-interacting protein 1 can interact with TrkB in neuronal cells, and can regulate the transport of TrkB in axons, but not in dendrites.

Key Words: *nerve regeneration; c-Jun NH2-terminal kinase-interacting protein; neurons; brain-derived neurotrophic factor; tropomyosin-related kinase B; axons; hippocampus; dendrites; regulation; neural regeneration*

Introduction

Proteins can be synthesized in neuronal cell bodies, and such proteins play important roles in dendrites and axons (Tan et al., 2010; Plazas et al., 2013). Accuracy of protein transport from the cell body to synaptic terminals directly affects the integrity of neuronal function. Brain-derived neurotrophic factor (BDNF) plays an important role in synaptic function. BDNF binds to tropomyosin-related kinase B (TrkB), and activated TrkB causes a series of neuron responses affecting the targets of such neurons (Nakaji-Hirabayashi et al., 2009; Sánchez-Migallón et al., 2011; Parkhurst et al., 2013a). Theoretically, after TrkB is synthesized, it is immediately transported to axon terminals, where it plays an important role in BDNF signal transmission. c-Jun NH2-terminal kinase (JNK)-interacting proteins (JIPs) are a series of JNK isoforms. Huang et al. (2011) suggested that JIP3 plays an important role in TrkB anterograde axonal transport. However, whether JIP1 mediates similar effects or precisely regulates TrkB anterograde axonal transport remains poorly understood. This study sought to analyze the regulatory effect of JIP1 on TrkB anterograde axonal transport in hippocampal neurons, and aimed to provide new data on the function of JIP1.

Materials and Methods

Ethics statement

This study was approved by the Experimental Animal Ethics Committee of the Experimental Animal Center, Zhengzhou University of China. Animal studies were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Precautions were taken to minimize suffering and the number of animals used in each experiment.

Animals

Sprague-Dawley rats aged 8–10 weeks and weighing 220–290 g (mean 273.7 ± 10.4 g) at gestational day 18 were purchased from the Experimental Animal Center of Zhengzhou University of China (animal license No. SCXK(Yu)2010-0002). All rats were allowed free access to food and water, and housed at 20–22°C in a 12-hour light/dark cycle. The rats were allowed to acclimate for 1 week.

Hippocampal neuron culture

After rats were sacrificed, the brains of embryos at embryonic day 18 were obtained. The hippocampi were isolated under an inverted microscope (TS100; Nikon, Tokyo, Japan) (Paxinos and Watson, 2005). Hippocampi were cut into blocks, and digested with 0.05% trypsin-ethylenediamine tetraacetic acid (EDTA) solution at 37°C for 15–20 minutes. The digestive waste was discarded. All samples were then incubated with Dulbecco's modified Eagle's medium (DMEM)/F12 media (Shanghai BioSun Sci&Tech Co., Ltd., Shanghai, China), triturated and deposited for 2 minutes. After removal of supernatant, samples were triturated into a single cell suspension, quantified using a hemocytometer (Shanghai Huake Experimental Equipment Co., Ltd., Shanghai, China), and diluted according to the density of the single cell suspension (5×10^6/mL). All samples were seeded onto a polylysine-coated culture dish at 37°C, 5% CO_2, and saturated humidity.

Passaging and culture of HEK293 cells

HEK293 cells (ATCC, Manassas, VA, USA) were rapidly defrosted, incubated with DMEM/F12 media, triturated, and mixed. Cell suspensions were incubated in the culture dish at 37°C, 5% CO_2, and saturated humidity. At 2 days after culture, cells reached 80% confluency and began to subculture. The medium was discarded. After washing with PBS, cells were digested with EDTA-PBS solution (0.2 mM) for 5 minutes. After the digestive waste was discarded, cells were incubated with HEK293 media, triturated into a single cell suspension, and centrifuged at 1,000 r/min for 2 minutes. After the supernatant was discarded, samples were incubated with cell media and triturated into a single cell suspension. The passage ratio was 1:3. Cells were then incubated in three culture dishes at 37°C, 5% CO_2, and saturated humidity.

Plasmid construction

pcDNA3.1 (Life Technologies, Carlsbad, CA, USA) was used as a vector. Flag-labeled TrkB and HA-labeled JIPA (Shanghai Sangon Biological Technology Co., Ltd., Shanghai, China) were made, and sequencing was conducted. Interfering RNA expression was initiated using the H1 promoter. The JIP1 sequence was 5′-GCG TCA GCG TCG AGT TTA A-3′.

Transfection

HEK293 cell suspension (5×10^6) was centrifuged in a centrifuge tube at 1,000 r/min for 5 minutes. The supernatant was discarded. 100 μL of electrotransfer buffer (2.5 μg/μL) was added to the cell suspension, triturated, and mixed. The mixture was placed in an electric rotor (nerve cells O-003 and HEK293 cells A-023). After electroporation, 500 μL of media was added and placed in a centrifuge tube in a 37°C water bath for 10 minutes. These cells were incubated in media at 37°C, 5% CO_2, and saturated humidity for 6 hours. The plasmids were than transfected.

Immunoprecipitation of proteins

Cultured cells were removed from the media, washed twice with PBS, and lysed with TNE buffer containing phosphatase and protease inhibitors. Lysed cells were scraped with a cell scraper, placed into a centrifuge tube, and lysed with a silent mixer (Kylin-Bell Lab Instruments Co., Ltd., Haimen, Jiangsu Province, China) for 30 minutes, and centrifuged at 25,000 × *g* for 10 minutes. Part of the cell lysate was uniformly mixed with an equal volume of 4× loading buffer. The mixture was heated at 98°C for 5 minutes. Mouse anti-rat HA monoclonal antibody, rabbit anti-rat Flag monoclonal antibody, rabbit anti-rat IgG monoclonal antibody, rabbit anti-rat TrkB monoclonal antibody, and mouse anti-rat JIP1 monoclonal antibody (1:1,000; GenScript Co., Ltd., Nanjing, China) were added to the remaining cell lysate in the silent mixer at 37°C for 2 hours. According to the primary antibody source, Protein A- or Protein G-coupled beads (secondary antibody; Beyotime Biotechnology, Shanghai, China) were added to the silent mixer at 4°C overnight. The mixture was centrifuged at 25,000 × *g* for 3 minutes, and the supernatant was discarded. TNE buffer was added to suspend the precipitate beads in triplicate. An equal volume of 4× loading buffer was added. Proteins were boiled at 98°C for 5 minutes, and experimental results were observed by electrophoresis.

Western blot assay

A premade plastic frame was placed into an electrophoresis tank, and an appropriate amount of buffer was added. Immunoprecipitation protein and cell lysate were added in the plastic frame in order. All samples were electrophoresed at a constant current for 1 hour, and transferred at 110 V for 2 hours. Polyvinylidene fluoride membrane was incubated with mouse anti-rat HA monoclonal antibody, rabbit anti-rat Flag monoclonal antibody, rabbit anti-rat IgG monoclonal antibody, rabbit anti-rat TrkB monoclonal antibody, and mouse anti-rat JIP1 monoclonal antibody (1:10,000; GenScript) at 4°C overnight. The membrane was washed three times with Tris-Buffered Saline and Tween 20 (TBST), and then treated with TBST-diluted horseradish peroxidase-goat anti-rabbit/mouse IgG (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at room temperature for 2 hours, followed by three washes with TBST.

Immunocytochemical staining

In accordance with a previously published staining method (Tang et al., 2011), samples were observed under the inverted fluorescence microscope (Nikon). Images were obtained by cold light sources (XD-301; RWD Life Science, Shenzhen, China), and analyzed using MetaMorph (Universal Imaging Corp, Downingtown, PA, USA). Dendrites were distinguished

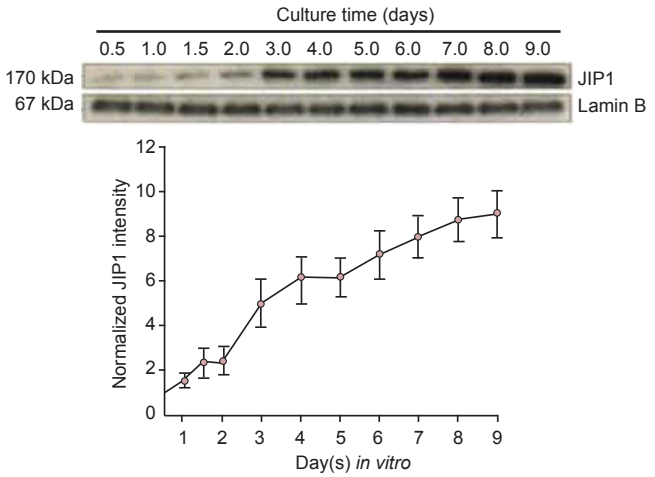

Figure 1 JIP1 expression in hippocampal neurons (western blot assay).
Data are expressed as the mean ± SD. Experiments were performed in triplicate. Intergroup comparisons were conducted using paired *t*-tests. JIP1: c-Jun NH2-terminal kinase-interacting protein 1.

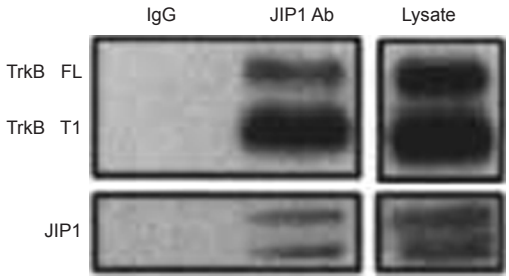

Figure 4 Interaction of JIP1 and TrkB in axons.
TrkB was precipitated by endogenous JIP1 antibody, suggesting that JIP1 could form a complex *in vivo*. FL: TrkB receptor subtype 1; T1: TrkB receptor subtype 2; Ab: endogenous JIP1 antibody; JIP1: c-Jun NH2-terminal kinase-interacting protein 1; TrkB: tropomyosin-related kinase B.

from axons based on morphology (Chang et al., 2014; Liu et al., 2014): dendrites were cone-shaped and thin, with irregular contour; axons were uniformly distributed, with flat contour and long processes. MetaMorph software was used to observe the gray value of TrkB-positive expression in the distal 30 μm of axons and the distal 10 μm of dendrites. The ratio of distal TrkB gray values to cell body values represents the relative distribution of TrkB. Experiments were performed in triplicate. Axons, in the third developmental stage (7–9 days of culture), were categorized into three parts: the proximal end, central, and distal end. The relative expression of JIP1 in each segment of the axon was calculated.

Statistical analysis

Data were analyzed using SPSS 16.0 software (SPSS, Chicago, IL, USA), and are expressed as the mean ± SD. Differences among groups were compared using paired *t*-tests. $P < 0.05$ was considered statistically significant.

Results

JIP1 expression in hippocampal neurons

Western blot assay results demonstrated that JIP1 expression significantly increased in hippocampal neurons at 2–4 days of culture (*vs.*, pre-culture, $P < 0.05$; **Figure 1**). This result suggested that 2–4 days of culture was a key stage in axon growth.

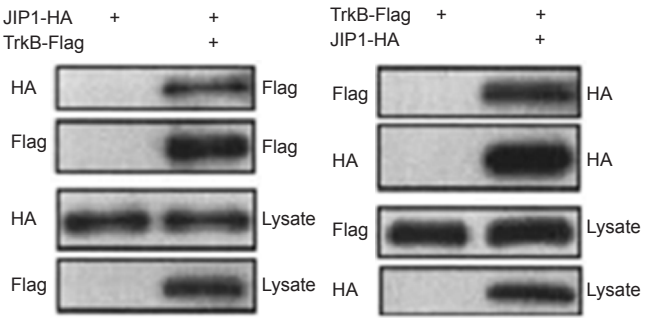

Figure 3 Interaction of JIP1 and TrkB *in vitro*.
JIP1-labeled HA and TrkB-labeled Flag were precipitated by corresponding molecules, suggesting that JIP1 could form a complex with TrkB. Flag: Labeled plasmid; HA: rabbit monoclonal antibody. JIP1: c-Jun NH2-terminal kinase-interacting protein 1; TrkB: tropomyosin-related kinase B.

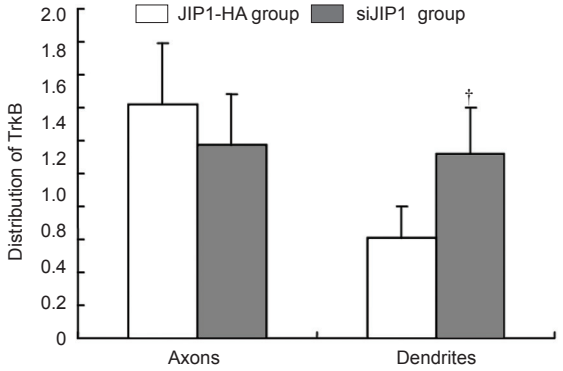

Figure 5 The effects of JIP1 on TrkB distribution in axons and dendrites (immunocytochemistry).
The ratio of gray values of TrkB in axons/dendrites to values in cell bodies represents the relative distribution of TrkB. Data are expressed as the mean ± SD. Experiments were performed in triplicate. Intergroup comparisons were conducted using paired *t*-tests. †$P < 0.05$, *vs.* JIP1-HA group. JIP1: c-Jun NH2-terminal kinase-interacting protein 1; TrkB: tropomyosin-related kinase B.

Distribution of JIP1 in the hippocampus

Immunocytochemical staining demonstrated that in 1–3 days of development, JIP1 could be detected in cell bodies and processes. In 4–9 days, JIP1 gradually congregated in the longest processes (**Figure 2**). At this time, processes already displayed the rudimentary features of axons. In 7–9 days, JIP1 expression was significantly higher in the distal end compared with the proximal end ($P < 0.05$; **Figure 2**).

Interaction of JIP1 and TrkB

Immunoprecipitation was performed in plasmids encoding JIP1-HA and TrkB-Flag using HA and Flag antibodies. Results revealed that JIP1-labeled HA and TrkB-labeled Flag could be co-precipitated by corresponding molecules, indicating that highly expressed JIP1 could form a complex with TrkB (**Figure 3**).

Using immunoprecipitation of endogenous proteins, JIP1 endogenous antibody was added to hippocampal lysates. Corresponding IgG antibody served as a negative control. Endogenous TrkB antibody was used for detection. Results

demonstrated that TrkB could be precipitated, indicating that JIP1 could form a complex *in vivo* (**Figure 4**).

The effects of JIP1 on the distribution of TrkB in axon terminals

Immunocytochemistry showed that the distribution of TrkB was significantly increased in axon terminals when JIP1 was highly expressed. After JIP1 knockout, the distribution of TrkB was significantly decreased in axon terminals. The difference in the distribution of TrkB remained significant whether JIP1 was knocked out or not ($P < 0.05$). However, in dendrites, JIP1 knockout did not affect the distribution of TrkB ($P > 0.05$; **Figure 5**).

Discussion

TrkB is a high affinity receptor for BDNF. TrkB is mainly distributed over neuronal cell bodies, dendrites, and axon terminals. Zhai et al. (2011) verified that the precise location of TrkB is important for normal nerve function in highly differentiated neurons. Presynaptic TrkB receptors can promote the release of glutamate and increase the activity of the Na^+/H^+ antiporter on target tissues (Numakawa et al., 2009). Postsynaptic TrkB receptors can enhance N-methyl-D-aspartic acid receptor function in central neurons. Thus, the precise location and correct transport of TrkB receptors is important in propagating the correct response to BDNF release. The CRMP-2/S1p1/Rab27B complex has been shown to mediate TrkB and affect kinesin-1 to regulate TrkB transport in axons (Lähteinen et al., 2004; Butowt and von Bartheld, 2007; Arimura et al., 2009; Poopalasundaram et al., 2011). When the CRMP-2/S1p1/Rab27B complex was knocked out, TrkB transport to synapses could not be completely blocked. This finding confirmed that other adapter proteins are likely involved in axon TrkB transport. Gatto et al. (2013) confirmed that TrkB transport from cell bodies to dendrites or axons occurs *via* a complex and precise regulatory mechanism. JIP3 can mediate the interaction of kinesin-1 light chain and TrkB, and regulate anterograde axonal transport of TrkB (Kanato et al., 2009; Arimoto et al., 2011; Fu and Holzbaur, 2013; Parkhurst et al., 2013b; Sun et al., 2013; Kim et al., 2014). However, the mechanism regulating TrkB is complex in neuronal cells. Additionally, other subtypes of the JNK family are possibly involved.

JIP1 was the first subtype of the JNK family to be discovered. Exogenous application of JIP1 can restore neurological function in mice with disconnection of the telencephalon induced by JIP3 knockout (Hayashi et al., 2007; Fujieda and Sasaki, 2008; Yamanaka et al., 2008; Nakaji-Hirabayashi et al., 2009). These results suggest that JIP1 and JIP3 have common features. Whether JIP1 has the same effect on mediating anterograde axonal transport of TrkB as JIP3 remains poorly understood. The present study first verified that JIP1 can form a complex with TrkB *in vivo* and *in vitro*, indicating that JIP1 potentially plays an important role in anterograde axonal transport of TrkB. The JIP3 and CRMP-2/S1p1/Rab27B complex pathways involved in regulating anterograde axonal transport of TrkB are completely different (Kim et al., 2004; Roberts et al., 2006; Bogush et al., 2007; Burkhalter et al., 2007; Caldeira et al., 2007; Zhang et al., 2007; Yamanaka et al., 2008). Thus, JIP1 may mediate anterograde axonal transport of TrkB, and in-turn, TrkB is a receptor involved in multiple regulatory pathways. Whether there is a link between these mechanisms is unconfirmed.

To further verify whether JIP1 has a similar role in dendrites and axons, we overexpressed JIP1-HA and JIP1 knockout in cultured neuronal cells *in vitro*. Immunocytochemistry showed that when JIP1 was highly expressed, the distribution of TrkB apparently increased in axon terminals. After endogenous JIP1 knockout, the distribution of TrkB was clearly reduced in axon terminals. Nevertheless, the distribution of TrkB was not associated with JIP1 expression in the ends of dendrites. This finding indicates that JIP1 only affected TrkB transport in axon terminals, minimally affected the distribution of TrkB in dendrites, and further verified that JIP1 exerted an effect on anterograde axonal transport of TrkB.

In conclusion, JIP1 may interact with TrkB in neuronal cells and regulate anterograde axonal transport of TrkB. Interestingly, the regulatory effect of JIP1 was exerted only in axons. This study demonstrated the effects of JIP1 on anterograde axonal transport of TrkB, but the precise mechanism and whether other proteins or molecules are involved during transport requires further investigation.

Author contributions: *All authors designed and evaluated the study, performed the experiments and approved the final version of the paper. ST wrote the paper.*
Conflicts of interest: *None declared.*
Plagiarism check: *This paper was screened twice using Cross-Check to verify originality before publication.*
Peer review: *This paper was double-blinded and stringently reviewed by international expert reviewers.*

References

Arimoto M, Koushika SP, Choudhary BC, Li C, Matsumoto K, Hisamoto N (2011) The Caenorhabditis elegans JIP3 protein UNC-16 functions as an adaptor to link kinesin-1 with cytoplasmic dynein. J Neurosci 31:2216-2224.

Arimura N, Kimura T, Nakamuta S, Taya S, Funahashi Y, Hattori A, Shimada A, Ménager C, Kawabata S, Fujii K, Iwamatsu A, Segal RA, Fukuda M, Kaibuchi K (2009) Anterograde transport of TrkB in axons is mediated by direct interaction with Slp1 and Rab27. Dev Cell 16:675-686.

Bogush A, Pedrini S, Pelta-Heller J, Chan T, Yang Q, Mao Z, Sluzas E, Gieringer T, Ehrlich ME (2007) AKT and CDK5/p35 mediate brain-derived neurotrophic factor induction of DARPP-32 in medium size spiny neurons in vitro. J Biol Chem 282:7352-7359.

Burkhalter J, Fiumelli H, Erickson JD, Martin JL (2007) A critical role for system A amino acid transport in the regulation of dendritic development by brain-derived neurotrophic factor (BDNF). J Biol Chem 282:5152-5159.

Butowt R, von Bartheld CS (2007) Conventional kinesin-I motors participate in the anterograde axonal transport of neurotrophins in the visual system. J Neurosci Res 85:2546-2556.

Caldeira MV, Melo CV, Pereira DB, Carvalho R, Correia SS, Backos DS, Carvalho AL, Esteban JA, Duarte CB (2007) Brain-derived neurotrophic factor regulates the expression and synaptic delivery of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunits in hippocampal neurons. J Biol Chem 282:12619-12628.

Chang X, Fang SH, Zhang Y, Yan R, Qu Z, Hou XQ, Su RY, Zhang L, Yang C, Wang Q (2014) Culturing primary hippocampal neurons of neonatal mouse and morphologic observation. Chongqing Yixue 43:2910-2912.

Fu MM, Holzbaur EL (2013) JIP1 regulates the directionality of APP axonal transport by coordinating kinesin and dynein motors. J Cell Biol 202:495-508.

Fujieda H, Sasaki H (2008) Expression of brain-derived neurotrophic factor in cholinergic and dopaminergic amacrine cells in the rat retina and the effects of constant light rearing. Exp Eye Res 86:335-343.

Gatto G, Dudanova I, Suetterlin P, Davies AM, Drescher U, Bixby JL, Klein R (2013) Receptor tyrosine phosphatase PTPRO inhibits trigeminal axon growth and branching by repressing TrkB and Ret signaling. J Neurosci 33:5399-5410.

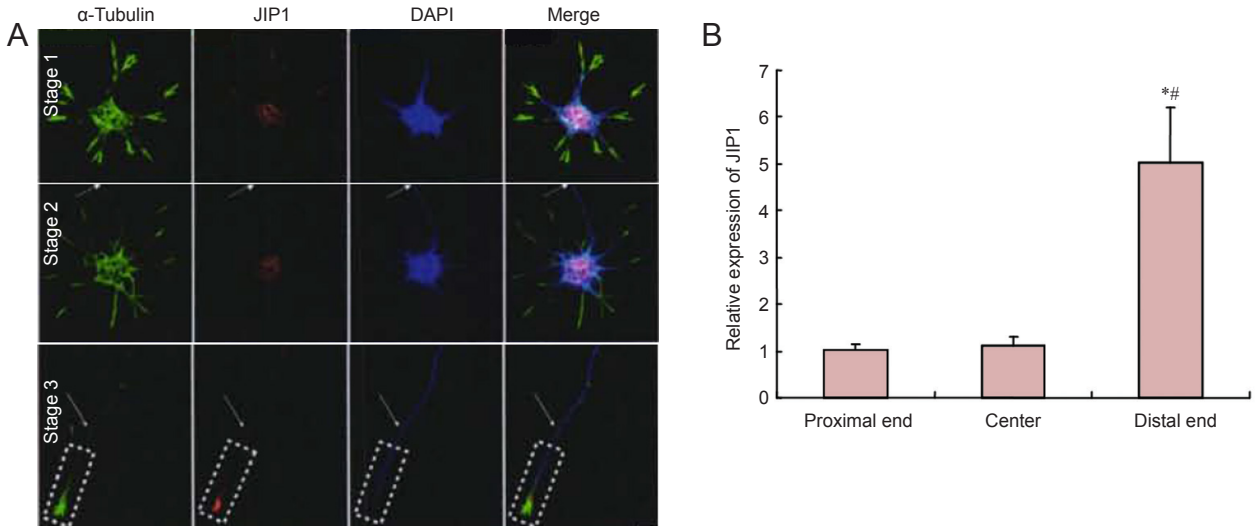

Figure 2 Relative expression of JIP1 in the hippocampus.
(A) JIP1 expression in different axonal developmental stages (immunofluorescence staining), × 400. Stage 1: 1–3 days of culture; stage 2: 4–6 days of culture; stage 3: 7–9 days of culture. Arrows show axons. Pane: Localization of JIP1. JIP1 is the red color, and α-tubulin is the green color. (B) JIP1 expression in different locations in stage 3. Data are expressed as the mean ± SD. Experiments were performed in triplicate. Intergroup comparisons were conducted using paired *t*-tests. **P* < 0.05, *vs.* the proximal end; #*P* < 0.05, *vs.* the center. JIP1: c-Jun NH2-terminal kinase-interacting protein 1.

Hayashi A, Kasahara T, Iwamoto K, Ishiwata M, Kametani M, Kakiuchi C, Furuichi T, Kato T (2007) The role of brain-derived neurotrophic factor (BDNF)-induced XBP1 splicing during brain development. J Biol Chem 282:34525-34534.

Huang SH, Duan S, Sun T, Wang J, Zhao L, Geng Z, Yan J, Sun HJ, Chen ZY (2011) JIP3 mediates TrkB axonal anterograde transport and enhances BDNF signaling by directly bridging TrkB with kinesin-1. J Neurosci 31:10602-10614.

Kanato Y, Ono S, Kitajima K, Sato C (2009) Complex formation of a brain-derived neurotrophic factor and glycosaminoglycans. Biosci Biotechnol Biochem 73:2735-2741.

Kim H, Li Q, Hempstead BL, Madri JA (2004) Paracrine and autocrine functions of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in brain-derived endothelial cells. J Biol Chem 279:33538-33546.

Kim YG, Kim JW, Pyeon HJ, Hyun JK, Hwang JY, Choi SJ, Lee JY, Deák F, Kim HW, Lee YI (2014) Differential stimulation of neurotrophin release by the biocompatible nano-material (carbon nanotube) in primary cultured neurons. J Biomater Appl 28:790-797.

Lähteinen S, Pitkänen A, Knuuttila J, Törönen P, Castrén E (2004) Brain-derived neurotrophic factor signaling modifies hippocampal gene expression during epileptogenesis in transgenic mice. Eur J Neurosci 19:3245-3254.

Liu J, Wang XY, Sun Y, Guo XY, Pang Y (2014) Morphological changes and NOS expression of neurons in CA3 hippocampus of rats. Jiepou Xuebao 45:181-184.

Nakaji-Hirabayashi T, Kato K, Iwata H (2009) Hyaluronic acid hydrogel loaded with genetically-engineered brain-derived neurotrophic factor as a neural cell carrier. Biomaterials 30:4581-4589.

Numakawa T, Kumamaru E, Adachi N, Yagasaki Y, Izumi A, Kunugi H (2009) Glucocorticoid receptor interaction with TrkB promotes BDNF-triggered PLC-γ signaling for glutamate release via a glutamate transporter. Proc Natl Acad Sci U S A 106:647-652.

Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR, Lafaille JJ, Hempstead BL, Littman DR, Gan WB (2013a) Microglia promote learning-dependent synapse formation through BDNF. Cell 155:1596-1609.

Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR, Lafaille JJ, Hempstead BL, Littman DR, Gan WB (2013b) Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell 155:1596-1609.

Paxinos G, Watson C (2005) The Rat Brain in Stereotaxic Coordinates. London: Academic Press.

Plazas PV, Nicol X, Spitzer NC (2013) Activity-dependent competition regulates motor neuron axon pathfinding via PlexinA3. Proc Natl Acad Sci U S A 110:1524-1529.

Poopalasundaram S, Marler KJ, Drescher U (2011) EphrinA6 on chick retinal axons is a key component for p75(NTR)-dependent axon repulsion and TrkB-dependent axon branching. Mol Cell Neurosci 47:131-136.

Roberts DS, Hu Y, Lund IV, Brooks-Kayal AR, Russek SJ (2006) Brain-derived neurotrophic factor (BDNF)-induced synthesis of early growth response factor 3 (Egr3) controls the levels of type A GABA receptor alpha 4 subunits in hippocampal neurons. J Biol Chem 281:29431-29435.

Sánchez-Migallón MC, Nadal-Nicolás FM, Jiménez-López M, Sobrado-Calvo P, Vidal-Sanz M, Agudo-Barriuso M (2011) Brain derived neurotrophic factor maintains Brn3a expression in axotomized rat retinal ganglion cells. Exp Eye Res 92:260-267.

Sun T, Yu N, Zhai LK, Li N, Zhang C, Zhou L, Huang Z, Jiang XY, Shen Y, Chen ZY (2013) c-Jun NH2-terminal kinase (JNK)-interacting protein-3 (JIP3) regulates neuronal axon elongation in a kinesin- and JNK-dependent manner. J Biol Chem 288:14531-14543.

Tan ZJ, Peng Y, Song HL, Zheng JJ, Yu X (2010) N-cadherin-dependent neuron–neuron interaction is required for the maintenance of activity-induced dendrite growth. Proc Natl Acad Sci U S A 107:9873-9878.

Tang S, Shen XY, Huang HQ, Xu SW, Yu Y, Zhou CH, Chen SR, Le K, Wang YH, Liu PQ (2011) Cryptotanshinone suppressed inflammatory cytokines secretion in RAW264.7 macrophages through inhibition of the NF-κB and MAPK signaling pathways. Inflammation 34:111-118.

Yamanaka M, Itakura Y, Ono-Kishino M, Tsuchida A, Nakagawa T, Taiji M (2008) Intermittent administration of brain-derived neurotrophic factor (BDNF) ameliorates glucose metabolism and prevents pancreatic exhaustion in diabetic mice. J Biosci Bioeng 105:395-402.

Zhai J, Zhou W, Li J, Hayworth CR, Zhang L, Misawa H, Klein R, Scherer SS, Balice-Gordon RJ, Kalb RG (2011) The in vivo contribution of motor neuron TrkB receptors to mutant SOD1 motor neuron disease. Hum Mol Genet 20:4116-4131.

Zhang X, Mei Y, Liu C, Yu S (2007) Effect of transcranial magnetic stimulation on the expression of c-Fos and brain-derived neurotrophic factor of the cerebral cortex in rats with cerebral infarct. J Huazhong Univ Sci Technolog Med Sci 27:415-418.

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